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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Toxicology Chapter for the Brodifacoum RED

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DP Barcode: D231157 Submission: S511727

Chemical: 112701 Brodifacoum

Attached is the Toxicology Chapter for the Brodifacoum RED. I have attempted to provide as much detail as possible, with particular emphasis on the high toxicity of this compound, its long half-life within the body, and the available information we have from different studies as to clotting times after exposure.

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B. Human Health Assessment

1. Hazard Identification

Brodifacoum is an anticoagulant rodenticide with terrestrial and residential non-food uses. The data requirements for this chemical are similar to those of the other anticoagulant rodenticides. At present, the available toxicological database for brodifacoum is adequate and will support reregistration.

Table 1. Required Toxicology Studies and Submitted Studies Satisfying the Data Requirements.

G. No.	Study Type	MRID No.	Satisfied
81-1	Acute oral-rat	42687501	Yes
81-2	Acute dermal	42232101	Yes
81-3	Acute inhalation toxicity	43110501	Yes
81-4	Primary Eye irritation	00066938	Yes
81-5	Primary Dermal irritation	--------	(not needed because of high toxicity)
81-6	Dermal Sensitization (0.25% formulation)	44021704	yes
82-2	21-day dermal toxicity	--------	no*
83-3a	Developmental toxicity-rat	00052443 40307202 42641902 92195013	Yes
83-3b	Developmental toxicity-rabbit	00052442 40307201	Yes
	Mutagenicity studies		
84-2	Ames assay	41563301	Yes
84-2	In vivo cytogenetics	41563302	Yes
	Special studies		
85-1	Metabolism study	00080235 42007502 44021705	Yes
86-1	Antidotal treatment study - dog	42007501	Yes

* This study is required.

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a. Acute Toxicity

Results of the acute toxicity studies conducted with technical brodifacoum are summarized below in Table 1:

Table 2. Acute Toxicity Values of Technical Brodifacoum.

Route	Species	Results	Toxicity Category
Oral	Rat	LD_{50} (M) = 0.418 mg/kg LD_{50} (F) = 0.561 mg/kg	I
Dermal	Rabbit	LD_{50} (M) = 5.21 mg/kg LD_{50} (F) = 3.16 mg/kg	I
Inhalation	Rat	LC_{50} (M) = 4.86 μg/L LC_{50} (F) = 3.05 μg/L	I
Eye Irritation[a]	Rabbit	Some minor eye irritation, clearing by day 7.	III
Skin Irritation[a]	Rabbit	Unlikely to cause anything more than mild irritation; the high toxicity (note the dermal LD_{50} values above) precludes necessity for testing the technical for dermal irritation potential.	-
Dermal Sensitization[a,b]	Guinea Pig	Non sensitizer	N/A

[a] Not required for TGAI, however, presented here for informational purposes.
[b] Conducted on the 0.25% Brodifacoum Formulation Concentrate; see below.

In an oral LD_{50} study in which technical brodifacoum (96.1%) was administered as a suspension in polyethylene glycol 300 to rats, there were no mortalities or signs of toxicity in males or females at 0.25 mg/kg, nor in males at 0.35 mg/kg (females were not tested at this dose level). However, 5/5 males and 1/5 females died following dosage at 0.5 mg/kg, and 5/5 females died following dosage at 0.75 mg/kg (males were not tested at this dose level). Signs of toxicity at 0.5 and 0.75 mg/kg included pallor, bleeding from the nose and/or rectum and/or other sites. Deaths occurred in the period from 3-8 days after dosing. Post mortem examination of those animals which died or were sacrificed in extremis and/or showed signs of bleeding revealed the presence of free or clotted blood in the abdominal and/or thoracic cavity. Discoloration or pallor of a number of organs was also observed. These findings are consistent with the known

anticoagulant activity of brodifacoum. The LD_{50} is calculated to be 0.418 mg/kg for males (95% confidence interval between 0.35 and 0.5 mg/kg) and 0.561 mg/kg for females (95% confidence interval 0.472-0.667 mg/kg). These results place brodifacoum in Toxicity Category I (MRID 42687501) by the oral exposure route.

In a dermal LD_{50} study with rabbits brodifacoum technical (95.6%) was applied as a suspension in corn oil (500 mg/kg), olive oil (10 mg/kg), or polyethylene glycol 600 (1 mg/kg), with 24-hour occluded dermal exposure. At 500 mg/kg, all the males were killed in extremis on days 5-6, and all females between days 5 and 8. At 10 mg/kg, 4/5 males were found dead or were killed in extremis between days 7 and 11, and 5/5 females between days 6 and 8. The animals which died or were killed showed signs of extreme toxicity consistent with anticoagulant activity (pallor, bleeding/bruising, breathing abnormalities) immediately prior to death. There were practically no signs of skin irritation in any of the animals. The dermal LD_{50} of brodifacoum technical was calculated to be 5.21 mg/kg (95% confidence interval 1.95-13.8 mg/kg) for males, and 3.16 mg/kg (95% c.i. 1.00-10.00 mg/kg) for females. These results place technical brodifacoum in toxicity category I (MRID 42232101) in terms of dermal toxicity potential.

In an inhalation LC_{50} study in rats, groups of young adult Wistar-derived rats, 5/sex, were exposed (nose only) for 4 hours to aerosols of brodifacoum (96.1% a.i.) generated from an acetone solution. The mean particulate concentrations were 0.82, 1.88, and 4.96 μg/L; corresponding brodifacoum concentrations were 0.69, 1.72 and 4.40 μg/L. The mass median diameters were 0.80, 0.89 and 0.68 μm, and the geometric standard deviations were 3.09, 1.91 and 2.54, respectively. Animals were observed for 14 days after exposure. Mortalities (accompanied by symptoms consistent with anti-coagulant activity) occurred on days 4-6 in 3/5 males and 5/5 females exposed to highest concentration (4.96 μg/L). The inhalation LC_{50} for males = 4.86 μg/L (based on particulate concentration), and for females = 3.05 μg/L. Brodifacoum technical (96.1%) is in toxicity category I (inhalation LC_{50} at or below 50 μg/L) based on the LC_{50} values in both sexes (MRID 43110501).

In an eye irritation study in rabbits, aliquots of 100 mg technical Brodifacoum (92.5%) were instilled in the conjunctival sac of the left eye in each of 9 New Zealand white rabbits. Three of the rabbit eyes were irrigated for one minute with lukewarm tap water starting 30 seconds after instillation of the test material. In some of the rabbits, there was subsequent iritis and/or slight redness of the conjunctivae with slight chemosis and discharge; with all irritation clearing by day 7. Brodifacoum technical (92.5%) is in toxicity category III in terms of eye irritation potential (MRID 00066938). However, it is noted that because of the high toxicity of brodifacoum, absorption of any significant amount of the technical material by the ocular exposure route might result in mortality (and the animals in this study were followed for only 7 days after exposure). Technical brodifacoum is in toxicity category III in terms of its ocular irritation potential.

There are no dermal irritation studies on technical brodifacoum. Because of the relatively high toxicity, dermal exposure to undiluted (or mixtures containing a relatively high percentage of) technical brodifacoum would probably be fatal (the dermal LD_{50} of brodifacoum technical in rabbits is given above as 5.21 mg/kg for males, and 3.16 mg/kg for females).

Because of the high toxicity of technical brodifacoum, end-use products (mostly containing 0.005% brodifacoum) are usually manufactured from a formulation containing 0.25% brodifacoum. Results of the acute toxicity studies conducted with Brodifacoum Formulation Concentrate are summarized below in Table 2:

Table 3. Acute Toxicity Values of Brodifacoum Formulation Concentrate (0.25%)

Route	Species	Results	Toxicity Category
Oral	Rat	LD_{50} (M) = 163 mg/kg LD_{50} (F) = 152 mg/kg	II
Dermal	Rat[a]	LD_{50} (M) > 2000 mg/kg LD_{50} (F) > 2000 mg/kg	III
Skin Irritation	Rabbit	Test material stained the skin pink at application site, but no indication of an inflammatory response	IV
Dermal Sensitization	Guinea Pig	Evaluation complicated by pink staining at the application site, but no evidence of a sensitization response.	N/A

[a]Study conducted with rats; however, rabbits may be a more sensitive species

In an acute oral toxicity study (MRID No. 44021701), groups of fasted, young Alpk:APfSD (Wistar-derived) rats, 5/sex were given a single oral dose of Brodifacoum Formulation Concentrate (active ingredient: Brodifacoum: label declaration 0.25%; analytical concentration 0.259%) in deionized water at doses of 50, 200, or 500 mg/kg (males), and doses of 100, 150 or 200 mg/kg (females), and were subsequently observed for 14 days.

LD_{50} Males = 163 (95% C.I.: 97-275) mg/kg
Females = 152 (95% C.I.: 132-175) mg/kg
Combined = not reported

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY II based on the oral LD_{50} in both sexes.

Animals which died or which subsequently showed symptoms were generally normal through day 4; symptoms (decreased activity, pallor, piloerection, stains around nose) in some animals were observed only on the day of (or the day before) death. Some rats which were found dead had shown no previous signs of toxicity. Mortalities occurred 4-7 days after dosing. Necropsy findings in rats which died included pallor of the kidney, liver, lung, pancreas and spleen, and clotted and/or free blood in the thymus and/or thoracic cavity, consistent with the anticoagulant activity of brodifacoum. There were no consistent effects on body weight.

In an acute dermal toxicity study (MRID No. 44021702), a group of five male and two groups each with five female young adult Alpk:APfSD (Wistar-derived) rats received a single 24-hour occluded dermal exposure to 2000 mg/kg undiluted Brodifacoum Formulation Concentrate (active ingredient: Brodifacoum: label declaration 0.25%; analytical concentration 0.259%). At 24 hours the application site was cleansed with cotton swabs. In order to prevent ingestion of any residual material, rats were fitted with collars which were kept in place until day 4 for the males and first group of females, and throughout the observation period for the second group of females. The animals were observed for 14 days following removal of the occlusive dressings. 1/5 males and 2/10 females died on days 7-9 with symptoms consistent with anticoagulant activity; one of the dead females is reported to have chewed and partly removed the dressing.

Dermal LD_{50} Males > 2000 mg/kg
Females > 2000 mg/kg
Combined > 2000 mg/kg

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY III in terms of dermal toxicity potential, based on the dermal LD_{50} values in both sexes. It is noted that this study was conducted with rats, which may be a less sensitive species than rabbits, which are usually used in dermal toxicity studies.

Among the survivors, one female showed bruising at the application site on days 10-15. Necropsy findings (pallor of the brain, liver, lung, pancreas and/or spleen) for animals which were killed in extremis were consistent with anti-coagulant activity of brodifacoum. Survivors all gained weight.

In a primary dermal irritation study (MRID No. 44021703), a group of six female young adult rabbits (New Zealand white), weights ranging from 3940-4290 g, each received a single 4-hour occluded dermal exposure to 0.5 ml of undiluted Brodifacoum Formulation Concentrate (0.25% a.i.), with scoring for dermal irritation within the first hour after removal of the occlusive wrap, and at 1, 2 and 3 days. There was slight edema only in one rabbit, and that was within one hour following exposure, but the test material stained the skin pink at the application sites, preventing full assessment of erythema. However, subsequent histopathological examination of application and unexposed skin

sites showed no indications of an inflammatory response associated with exposure to the test material.

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY IV in terms of dermal irritation potential, based on the lack of any significant irritation (slight edema observed in only one animal within one hour following exposure, and lack of inflammatory response observed in histopathological examination.

In a dermal sensitization study (MRID 44021704) with Brodifacoum Formulation Concentrate (0.25% a.i.), administered at challenge undiluted and as 30% and 10% w/v suspensions in deionized water, young adult Crl:(HA)BR male guinea pigs were tested using the method of Buehler.

There were no indications of a sensitization reaction, although evaluation was complicated by pink staining at the application sites. Skin samples were examined histopathologically, with no indications of a significant inflammatory response. In this study, Brodifacoum Formulation Concentrate (0.25% a.i.) is not a dermal sensitizer.

b. Subchronic Toxicity

The Agency has no record that any subchronic toxicity studies on brodifacoum have been received and/or reviewed; however, it is noted that there are a number of multiple-dose studies that the Agency has received (including a special study (Brodifacoum: Blood Kinetics Study in the Pregnant Rat, MRID 42641902, see below), that include prothrombin time measurements which appear to be the most sensitive indicator of toxicity for the anticoagulants.

Because of the potential for non-purposeful dermal exposure, and to more accurately assess the margins of exposure associated with potential incidental exposure, a 21-day dermal toxicity study, (Guideline 82-2) is required (as confirmatory data; however, the current toxicological data base is sufficient for the purposes of this RED). **Such a study should include prothrombin time measurements, including pre-exposure, and for days 7, 14 and 21.**

c. Chronic Toxicity and Carcinogenicity

There are no chronic toxicity and/or carcinogenicity studies on brodifacoum. Given the exclusively non-food uses (and the negative findings in the mutagenicity studies), no chronic and/or carcinogenicity studies are required.

d. Developmental Toxicity

In a developmental toxicity study (MRID 00052443, with additional data in MRID 40307202) brodifacoum (92.5%) was administered to 30 Alderley Park, Wistar-derived

mated female rats/dose level by gavage in 10% v/v ethanol:water at dose levels of 0 (vehicle only), 0.001, 0.01 and 0.02 mg/kg/day from days 6 through 15 of gestation (as reported: in this study the morning on which spermatozoa were detected in vaginal smears was designated as day zero of gestation. In more recent studies the morning that sperm are detected is designated as day one of gestation).

There was blood in the uteri of one 0.01 and three 0.02 mg/kg females. This was considered to be possibly related to the administration of brodifacoum. There were no indications of any dose-related developmental effects associated with exposure to brodifacoum at doses up to and including 0.02 mg/kg/day. The dose level of 0.02 mg/kg/day is considered adequate, based on the occurrence of 100% mortality at a nominal value of 0.05 (analytical value of 0.35) mg/kg/day in a preliminary study, and blood measurements in a special study (Brodifacoum: Blood Kinetics Study in the Pregnant Rat, MRID 42641902, see below).

The rat maternal toxicity NOEL is 0.001 mg brodifacoum/kg/day (based on the equivocal finding of blood in the uteri of one 0.01 and three 0.02 mg/kg females).

The rat developmental NOEL is 0.02 mg brodifacoum/kg/day (HDT).

This developmental toxicity study in the rat is classified as Acceptable (Guideline) (83-3a), and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(a)) in the rat.

In a special study (MRID 42641902) in which mixtures of unlabeled brodifacoum (98.7%) and radiolabelled brodifacoum (radiochemical purity >95%) were administered to Alderley Park, Wistar-derived mated female rats by gavage at nominal doses of 0.0125 mg/kg (Group A: 24 rats, starting on day 1 of gestation, with sacrifice by exsanguination of 3 rats on days 1, 3, 5, 7, 9, 11, 13, 16) and 0.02 mg/kg (Group B: 15 rats, starting on day 7, with sacrifice of 3 rats on days 7, 9, 11, 13 and 16). The test material was administered as a suspension in polyethylene glycol 600. Terminal blood samples were analyzed for brodifacoum levels.

The following mean ng equivalents of brodifacoum/gram of maternal blood were observed: Group A (0.0125 mg/kg/day, days 0-16): day 1: 0.560; day 3: 0.924; day 5: 1.556; day 7: 1.809; day 9: 2.015; day 11: 2.795; day 13: 2.168; day 16: 3.396. Group B (0.02 mg/kg/day, days 7-16): day 7: 0.691; day 9: 1.362; day 11: 3.087; day 13: 2.427; day 16: 4.488.

The relative proportions of mean blood brodifacoum levels in group B rats as compared to group A rats were the following: Day 7: 0.382; Day 9: 0.666; Day 11: 1.10; Day 13: 1.12; and Day 16: 1.32.

In this study there was a steady increase of blood brodifacoum levels with continued dosage of both 0.0125 mg/kg/day and 0.02 mg/kg/day, consistent with findings of a previously reviewed metabolism study (MRID 00080235), in which three rats given a single oral dose of 0.25 mg labeled brodifacoum still retained a mean of 77.73% of the initial dose (mean total label recovery was 91.51%) after 10 days. The combination of high toxicity and body accumulation of brodifacoum would have eventually resulted in mortalities at these dosage levels at some time after 16 days.

The study is classified as Acceptable (Nonguideline) as it is not a required guideline study. It is acceptable for the purposes for which it was intended as a special study, and the findings adequately justify the dosing schedule and doses used in the rat developmental toxicity study (MRID 00052443 and 40307202; summarization in MRID 92195013).

In a developmental toxicity study in rabbits (MRIDs 00052442 and 40307201) brodifacoum (92.5%) was administered to 15 mated female Dutch rabbits/dose level by gavage in 5% v/v ethanol:water at dose levels of 0 (0.5% v/v aqueous Tween 80), 0 (5% v/v aqueous ethanol, the vehicle used with brodifacoum), 0.001, 0.002 and 0.005 mg brodifacoum/kg/day from days 6 through 18 of gestation (in this study gestation day 0 was the day of mating. In more recent studies it is designated as day one of gestation).

Ten of the 15 rabbits receiving 0.005 mg/kg/day died or were humanely killed; all were found to have internal hemorrhage. Nine of these does had loss of blood (in some cases heavy) from the vagina. All of the implants of one doe (#47; killed on day 16) in the 0.005 mg/kg/day group are reported to have had a hemorrhagic appearance, but otherwise there were no indications of any dose-related developmental or toxic effects associated with exposure to brodifacoum at doses up to and including 0.005 mg/kg/day. Because only three litters (and only 20 fetuses) were available from the 0.005 mg/kg/day group at 29 days (and taking into consideration the hemorrhagic appearance of the implants of #46), the NOEL for fetal toxicity is 0.002 mg/kg/day, and the LOEL is 0.005 mg/kg/day. The only possible indication of toxicity in the 0.002 mg/kg/day does was the occurrence of a small hemorrhage beneath the lid of one eye on gestation day 14 in one rabbit (#44) which was not pregnant, but a similar finding was not reported for the 0.005 mg/kg/day females.

In addition, the prothrombin time was significantly increased at 0.005 mg/kg/day on day 20 relative to controls (to 26.5 [seconds?] from 14.5) in a preliminary range-finding study.

The following table shows the prothrombin time measurements (presumably in seconds) on day 20 in a preliminary range-finding study.

Table 4. Prothrombin Time in the Preliminary Developmental Toxicity Range-Finding Study in the Rabbit (Day 20)

	Control	0.001 mg/kg/day	0.005 mg/kg/day
Mean	14.5	17.4	26.5**
SD	2.0	-	5.1
No. of samples	4	1	3

** Statistically significant at the 1% level (Student's t-test) compared with the control group
Data extracted from appendix 1 of MRID 00052442 (p. 31)

The rabbit maternal NOEL is 0.002 mg brodifacoum/kg/day. The LOEL is 0.005 mg/kg/day (based on 75% mortality associated with hemorrhage in pregnant females at this dose level). The developmental toxicity NOEL is 0.002 mg/kg/day, as only 3 litters (with a total of 20 fetuses) were available for evaluation at 0.005 mg/kg/day), and it is reported that all of the implants from a 0.005 mg/kg/day doe which was killed on day 16 had a hemorrhagic appearance.

This developmental toxicity study in rabbits is classified as Acceptable (Guideline) (83-3b).

e. Reproductive Toxicity

A 2-generation reproduction toxicity study is not required for brodifacoum as there are no food uses associated with this active ingredient, and there would not be any significant (in terms of frequency, magnitude, or duration) human exposure associated with its uses.

f. Mutagenicity

In a Salmonella typhimurium (Ames) assay which was conducted with and without metabolic activation (S9 from rat liver) in replicate studies with doses ranging from 1.6 - 5000 μg/plate in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538. Additional testing was carried out using a dose range of 0.064 - 200 μg/plate

with and without S9 in strains TA1538 and TA100. The test material was delivered to the test system in dimethyl sulfoxide. Compound insolubility was seen at 5000 μg/plate +/- S9. Cytotoxicity was observed for the majority of the strains at ≥40 μg/plate -S9 and ≥1000 μg/plate +S9.

In the first trial, there was a 14.6x increase in revertants (relative to the mean control value) for strain TA1538 at the highest dose level (5000 μg/plate, with precipitation of the test material) -S9; however, replicate plating indicated these colonies were not protrophic mutants and an increased incidence of revertants in this strain and at this dose level was not observed in the second trial. **It is concluded then that there was no evidence that brodifacoum induced a mutagenic response in any strain at any nonactivated or S9-activated dose level.** This study (MRID 41563301) satisfies the Guideline requirement (84-2) for a Salmonella typhimurium (Ames) reverse mutation assay.

In a mouse micronucleus assay (MRID 41563302), groups of five male and five female C57BL/6J mice received single intraperitoneal injections of 0.187 or 0.30 mg/kg Brodifacoum (96%) in corn oil; these doses represented 50 and 80% of the 7-day median lethal dose respectively. Mice were sacrificed at 24, 48 and 72 hours postadministration and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).

No deaths or other signs of toxicity were reported, and there was no evidence of target cell cytotoxicity; however, 70% of the mice administered 0.5 mg/kg in a preliminary study died. The positive control induced the expected high yield of MPEs in males and females. **Brodifacoum did not induce a clastogenic and/or aneugenic effect in either sex at any dose or sacrifice time.** This study (MRID 41563302) satisfies Guideline requirements (84-2) for an in vivo chromosomal aberration assay.

The two studies cited above (MRID 41563301 and 41563302) satisfy the mutagenicity data requirements for an anticoagulant. Overall, these results indicate brodifacoum has little, if any, genotoxic activity.

g. Metabolism

In the first part of a metabolism study (MRID 44021705) Brodifacoum, 3-[3-(4'-bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one, radiochemical purity >98%, radiolabelled (^{14}C) in the benzene ring of the benzopyran, was administered to 3 previously bile-duct cannulated Crl:CD(SD)BR strain male rats as a single oral administration at a nominal dose level of 10 mg/kg body weight, well above the LD_{50} value of 0.3 mg/kg. The rats had been predosed with vitamin K_1 in their drinking water, but showed symptoms of anticoagulant toxicity before sacrifice at 48 hours. Bile, urine and feces were collected at pre-dose, 6, 12, 24, and 48 hr post-dose, and radioactivity was determined in these samples, as well as in the livers

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and residual carcasses. The metabolite profiles of ^{14}C-brodifacoum in bile and bile extracts were examined by chromatographic and spectroscopic techniques.

Total mean recovery of radioactivity was 102.9 ± 8.1%. Recovery from feces (presumably unabsorbed brodifacoum) was 36.11 ± 8.83%; from liver was 14.79 ± 0.41; from the residual carcass: 42.85 ± 5.06%. The mean from bile (all 3 animals) was 6.40 ± 5.45%, but one rat had poor bile flow, possibly from blockage in the cannula; the two remaining animals had a mean 9.53% of the label in bile.

The major (and only identified) metabolite of brodifacoum in bile was the glucuronide (attachment to the 4-hydroxy moiety of brodifacoum), which accounted for 39.43 to 77.28% of the total radioactivity in individual bile samples, while brodifacoum represented 0.00 to 24.95% of the total activity. Further characterization appeared to split the glucuronide peak into 2 components, and while the cis:trans ratio of parent material was 70:30, the ratio in the glucuronide was reversed (30:70). One unidentified metabolite (region 10) ranged from 1.59 to 21.7% total radiolabel.

Although only one metabolite (the glucuronide) is identified, it is the parent compound which is of toxicological concern, and the registrant has adequately demonstrated in previously submitted studies (refer to MRIDs 00080235 and 42007502) that a high proportion of unmetabolized compound is retained, particularly in the liver.

In a second study (*in vitro* perfusion, also in MRID 44021705) the lower vena cava of a single male rat was ligated; the hepatic portal vein was then cannulated and the liver was cleared of blood and the bile duct cannulated. The liver was perfused, and, after equilibration, ^{14}C-brodifacoum, at a dose of 10 mg/kg, was added to the main perfusate reservoir; bile and perfusate were collected at pre-dose, 1 minute (perfusate only), 1, 2, 3, 4 and 6 hr post-dose. The radioactivity present in bile, perfusate, terminal perfusate supernatant, supernatant filtrate and liver was determined. There was 74.32% recovery after 6 hours, with 59% of the total in perfusate, and 15.19% in liver. Metabolite profiling was attempted, but no metabolites were identified. All radioactivity in the perfusate supernatant was bound to perfusate proteins, with no activity being measured in the aqueous filtrate.

In a metabolism study (MRID 42007502) groups of male rats received single oral doses of ^{14}C-labeled brodifacoum at different dose levels (Group 2: 0.02 mg/kg; Group 3: 0.15 mg/kg; Group 4: 0.35 mg/kg), and blood was taken from 1-3 rats/group at various intervals following this dosage. The following Kaolin Cephalin Time (KCT) and Prothrombin Time (PT) measurements were made:

Table 5. Kaolin Cephalin and Prothrombin Time in a Metabolism Study in Male Rats

	Group 2 0.02 mg/kg		Group 3 0.15 mg/kg		Group 4 0.35 mg/kg	
	Clotting times (seconds)		Clotting times (seconds)		Clotting times (seconds)	
Time after Dosing	KCT	PT	KCT	PT	KCT	PT
6 hr	-	-	-	-	ND	14.3 ± 1.7
12 hr	-	-	-	-	ND	20.7 ± 3.7
18 hr	-	-	-	-	43.7 ± 2.1	37.2 ± 5.4
24 hr	14.9[a] ±4.2	13.0[a] ±1.8	15.8 ± 4.8	13.0 ± 1.1	58.9 ± 7.6	95.5 ± 2.7
48 hr	-	-	-	-	113.7±10.6	147.6± 6.9
72 hr	-	-	-	-	92.8 ±49.4	39.7 ±19.4
96 hr	-	-	-	-	32.3 ± 7.2	18.8 ± 2.0
Day 8	-	-	-	-	21.3[a]± 2.4	15.8[a]± 1.2
Day 14	-	-	14.0 ± 1.1	14.3 ± 0.2	15.4 ± 4.5	17.4 ± 0.5
Day 28	14.9[a] ±1.1	12.7[a] ±0.3	21.3 ± 2.9	13.6 ± 0.6	20.2 ± 2.9	13.4 ± 0.4
Day 56	-	-	16.2[a]± 2.4	12.7[a]± 0.6	19.6[a]± 2.2	13.3[a]± 0.2
Day 84	-	-	-	-	17.2 ± 2.9	12.5 ± 0.4
Week 13	14.1[a] ±1.1	15.4[a] ±0.6	16.5 ± 1.4	13.8 ± 0.2	-	-
Week 26	-	-	12.3*	16.1*	-	-
Week 39	16.6 ± 4.3	13.5 ± 1.2	15.0 ± 1.7	13.8 ± 0.5	-	-
Week 52	-	-	15.6 ± 6.2	12.7 ± 1.2	-	-
Week 65	16.7 ± 3.3	13.5 ± 0.8	18.0 ± 3.2	13.2 ± 0.5	-	-
Week 78	-	-	18.6 ± 1.3	12.8 ± 1.2	-	-
Week 91	16.8 ± 2.0	14.6 ± 0.4	19.8 ± 2.2	15.1 ± 1.5	-	-
Week 104	14.7 ± 3.0	11.1 ± 1.0	13.2 ± 0.5	10.9 ± 0.6	-	-

The standard deviation (SD) is derived from data obtained with 3 animals per group.
* = single value only.
a = 2 values only
ND = not determined
Table taken from p. 26 of MRID 42007502.

The results given above clearly show an increase in clotting time in rats which had received a single oral dose of 0.35 mg/kg. Assuming the effect was manifested as a doubling of the normal clotting time (to approximately 30 seconds for kaolin cephalin and/or prothrombin times), effects were evident as soon as 18 hours after dosage, and were still present at 96 hours post-dosage.

In addition, the metabolism study in MRID 42007502 demonstrates that considerable amounts of the radiolabel are retained in the liver following dosage.

Table 6. Percentage of radioactivity retained in the liver following single-dose administration of ^{14}C Brodifacoum

Time after dosing	Group 2 (0.02 mg/kg) Mean SD	Group 3 (0.15 mg/kg) Mean SD	Group 4 (0.35 mg/kg) Mean SD
Day 1	47.33 ± 10.87	29.71 ± 4.40	28.92 ± 1.79
Week 4	39.16 ± 3.50	37.07 ± 1.94	23.47 ± 1.21
Week 8	-	30.86 ± 4.23	23.00 ± 0.09
Week 12	-	-	21.24 ± 3.19
Week 13	34.01 ± 2.49	31.74 ± 5.13	-
Week 39	20.33 ± 0.42	22.02 ± 2.83	-
Week 65	15.97 ± 2.33	15.36 ± 3.03	-
Week 91	10.57 ± 1.08	12.39 ± 3.08	-
Week 104	11.78 ± 0.97	11.74 ± 1.64	-

Table from data on pages 30-32 of MRID 42007502.

It is concluded that overall there is sufficient metabolism data (including excretion, distribution, retention half-life and amounts retained within different organs). This metabolism study in the rat then, when taken with previously submitted metabolism studies (in MRIDs 00080235 and 42007502) is classified as acceptable; and the combination of these studies is adequate to satisfy the 85-1 data (metabolism study) guideline requirement.

In an antidotal study (MRID 42007501) four male beagle dogs each received a single oral dose of 5 mg/kg brodifacoum (96.8%). Prothrombin times for each of the dogs were then monitored over a period of five weeks. "Doses of 2 mg/kg vitamin K_1 were administered to dogs by the intramuscular route whenever their prothrombin times were elevated to levels consistent with a life-threatening effect on coagulation." Individual dogs required 12-15 vitamin K_1 treatments in the period from days 2 to 29 post-dosing. All four dogs survived to the end of this study (5 weeks after the test material was administered). However, based on elevations in prothrombin time, vitamin K_1 was administered to one dog on day 29; this dog had previously received vitamin K_1 on days 23 and 24, and the last prothrombin time measurement for this dog was on day 34.

While vitamin K_1 has been shown to be an effective treatment following brodifacoum ingestion, there still remains the possibility of incidents involving pets or small children in which it is not known or realized that ingestion has occurred until it is too late for effective treatment. This possibility remains a concern to the Agency.

Examination of the accepted labels for a number of Brodifacoum-containing products indicates that although many include a statement similar to the following: "For Human Cases: Vitamin K_1 is antidotal at doses of 10-20 mg (not mg/kg). Repeated treatments may need to be given for up to 30 days (based on monitoring of prothrombin times)" others specify that: "For human cases, vitamin K_1 is antidotal... Repeated doses may need to be given up to two weeks (based on monitoring of prothrombin times)." Where appropriate, revisions should be made specifying monitoring of prothrombin times for at least 30 days after ingestion (and if prothrombin times are elevated at any point during this period, monitoring should be continued after 30 days).

2. Dose Response Assessment

a. Reference Dose

The HED Reference Dose (RfD)/Peer Review Committee evaluated the toxicological data on brodifacoum and recommended that no RfD be established.

b. Other Toxicological Endpoints

Since no dermal absorption data are available, 100% dermal absorption for brodifacoum is assumed. The toxicity endpoints for the acute dietary, short- and intermediate-term occupational risk assessments are based on the maternal and developmental toxicity NOEL of 2 μg/kg/day (LOEL = 5 μg/kg/day) observed in a developmental study in rabbits (MRIDs 00052442 and 40307201).

CHEMICAL: BRODIFACOUM
PC CODE: 112701

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